Targeting neural precursors in the adult brain rescues injured dopamine neurons

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In Parkinson's disease, multiple cell types in many brain regions are afflicted. As a consequence, a therapeutic strategy that activates a general neuroprotective response may be valuable. We have previously shown that Notch ligands support neural precursor cells in vitro and in vivo. Here we show that neural precursors express the angiopoietin receptor Tie2 and that injections of angiopoietin2 activate precursors in the adult brain. Signaling downstream of Tie2 and the Notch receptor regulate blood vessel formation. In the adult brain, angiopoietin2 and the Notch ligand Dll4 activate neural precursors with opposing effects on the density of blood vessels. A model of Parkinson's disease was used to show that angiopoietin2 and Dll4 rescue injured dopamine neurons with motor behavioral improvement. A combination of growth factors with little impact on the vasculature retains the ability to stimulate neural precursors and protect dopamine neurons. The cellular and pharmacological basis of the neuroprotective effects achieved by these single treatments merits further analysis.

neural stem cell | vascular system

A hallmark of Parkinson's disease (PD) is the progressive loss of the dopamine neurons that extend axons from the substantia nigra to the striatum and have a major role in the motor system. Grafts of dopamine neurons derived from embryonic (ES) and induced (iPS) cells are presented as an exemplar of the clinical potential of stem cell biology programs that are currently attracting major investments around the world (1–3). However, the value of cell therapy is questioned by data from transplant patients suggesting the grafted neurons have compromised function and acquire disease (4–6). The use of gene therapies that directly target dopamine neurons is being explored in preclinical models of PD (7). The identification of precursor cells in the adult nervous system raises the possibility that clinical benefit may be achieved indirectly by activating immature cells that are already present in the brain.

Scientific interest in precursors present in the adult brain has mostly focused on the subventricular zone (SVZ) and the dentate gyrus (DG) of the hippocampus, regions that give rise to new neurons. Here we define a widely distributed precursor by expression of the transcription factor Hes3. Hes3 is a member of the Hes/Hey gene family that mediates transcriptional responses to Notch activation and contributes to neuroepithelial development in mice (8). In neural stem cells, Notch activation of a self-renewal response promotes a rapid increase in the level of expression of Hes3 (9). In this study, we use Hes3 expression to show that this widely distributed precursor rapidly responds to single injections of signaling proteins.

Hes3-positive cells in the primate and rodent brain are closely associated with blood vessels and co-express Tie2. The Tie2 receptor for angiopoietins is known to regulate endothelial and hematopoietic stem cells (10–13). Delivery of either angiopoietin2 (Ang2) or the Notch ligand Dll4 into the adult brain causes an increase in Hes3-positive cells and opposing effects on the number of blood vessels over large areas of the central nervous system. A combination of growth factors including both Ang2 and Dll4 had no net effect on the number of blood vessels. Treatment with either single factors or a combination of factors rescued dopamine neurons from a cytotoxic injury and supported sustained behavioral improvement. The neuroprotection seen in these animals encourages further study of this widely distributed and readily activated response.

Results

Neural Precursors Express Tie2 in the Adult Brain. Tie2 is known to be expressed in neural tumors, but we know little about the normal role of this receptor in the adult brain. Well-characterized antibodies were used to demonstrate that Tie2 was expressed in the SVZ of adult rats, a region known to contain a high density of precursor cells expressing the SRY transcription factor Sox2 and the intermediate filament protein nestin (14) (Fig. 1*A*, inset). When ligand is bound, the Tie2 receptor becomes phosphorylated; an antibody that recognizes the activated receptor shows that many cells in the SVZ express active Tie2 (Fig. 1*A*).

pTie2/Sox2 double-positive cells were also found in the DG of the hippocampus (Fig. 1*B*). These cells were present in the subgranular zone (SGZ), where the precursor cells to granule neurons are found. In the hippocampus, only a subset of Sox2-positive cells co-express Tie2. The less dense distribution of precursor cells in the hippocampus permits optical sections to demonstrate that Tie2-positive cells contain a Sox2-positive nucleus (Fig. 1*C* and Fig. S1*a*). Optical sections of the rat striatum and corpus callosum show that Tie2-positive cells also express Hes3 (Fig. S1*b* and *c*). Extensive studies in cell culture define multipotent and differentiated states of neural precursors isolated from the fetal telencephalon (15). The use of 4 different antibodies against Tie2 shows that the undifferentiated cells express this protein at higher levels than their differentiated progeny. Neural precursors isolated from the fetal mouse neocortex and adult rat SVZ co-express Hes3, Sox2, and Tie2 in cell culture (Fig. S2*a–c*). Western blots also show that fetal neural precursors in vitro express Tie2 that can be phosphorylated by treatment with Ang2 in a time-dependent manner (Fig. S2*d*). Consistent with a precursor nature of Hes3-positive cells, Hes3 expression in fetal and adult neural precursors in culture is lost following a differentiation Fig. S3*a–d*). Ang2 treatment also induced phosphorylation of STAT3 on the serine 727 residue, a

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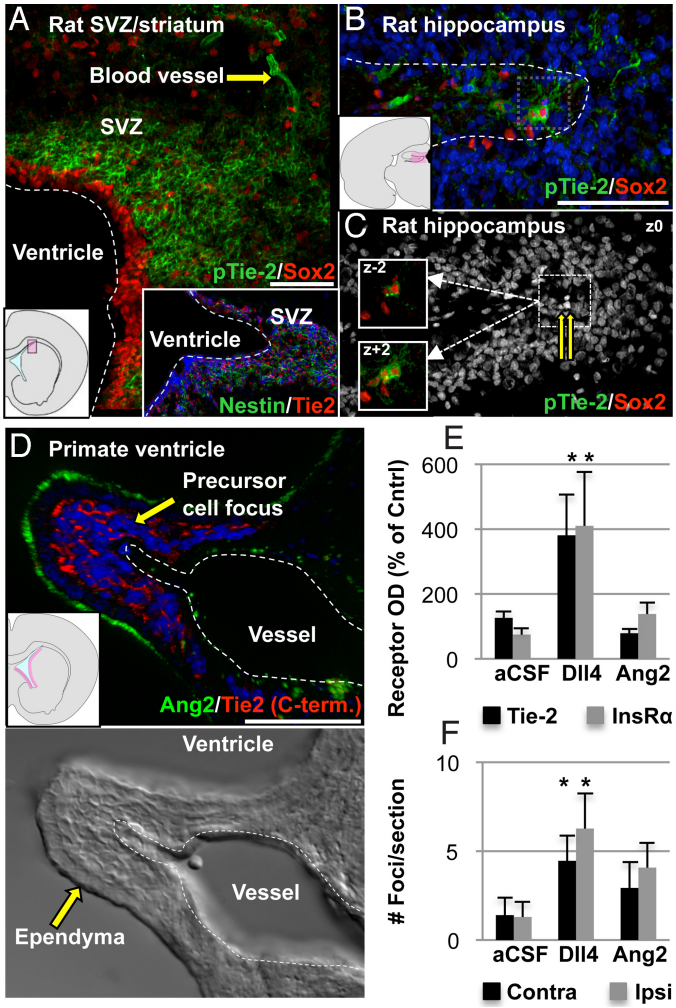

Fig. 1. Neural precursors in the adult brain express the Tie2 receptor. (*A*) Phosphorylated Tie2 (pTie2) is expressed in the SVZ and blood vessels of the adult rat brain (*Inset*, nonphospho Tie2 expression in the SVZ). (*B*) pTie2 is co-expressed with Sox2 in the SGZ of the DG of the adult rat hippocampus (image from a CT-treated animal). (*C*) DAPI counterstain of the image in *B* and 2 single z planes, 4 μm apart (arrows point to the Sox2- positive /pTie2-positive nuclei). (*D*) A single Dll4 injection in the monkey striatum induces the formation of Tie2-rich foci at the surface of the lateral ventricle (image from a Dll4-treated monkey; below, DIC image of the same area). (*E*) Quantitation of Tie2 and insulin receptor subunit a signal of adult monkeys 10 days after single striatal injections of factors (data are ipsilateral OD signal from digital immunohistochemistry images, expressed as % contralateral striatum). (*F*) Quantitation of foci number in the ipsilateral and contralateral hemispheres of adult monkeys [data are total number of foci per coronal section of the SVZ (30 μm thickness)]. (Scale bars, 50 μm.)

critical regulator of stem cell survival (9). These data suggest that the Tie2 receptor regulates neural precursors in vitro and in vivo.

In the rat and primate brain, Tie2-positive cells were also found in regions distant from the ventricle and the DG. Tie2- and Hes3-positive cells in the striatum of the Rhesus monkey (*Macaca mulatta*) are shown in Fig. S4*a* and *b*. Many of these Hes3-positive cells also express high levels of the insulin receptor (Fig. S4*c* inset). Insulin receptor was assessed as exogenous insulin has physiological and neurotrophic effects on dopamine neurons in the rat striatum (16, 17). These results show that Tie2 is expressed on a subset of cells in the adult brain that may respond to exogenous angiopoietin and other growth factors.

To explore the effects of Ang2 in vivo, convection delivery was used to introduce exogenous Ang2 or Dll4 into the striatum of the adult Rhesus monkey. In the primate, convection delivery has been optimized using simultaneous magnetic resonance imaging (MRI) to show that exogenous proteins are restricted to the brain region that is injected (18). The restricted delivery of protein to the ipsilateral striatum was confirmed by MRI in the animals used in this study. Ten days after protein was injected, both treatments induced the formation of foci of cells on the lateral and medial ventricular surfaces but not the ventricular surface lining the corpus callosum (Fig. 1*D* and Fig. S4*c*). This result is consistent with previous reports that exogenous mitogens promote the formation of aggregates of precursor cells that project into the ventricular space (19). Here we show that the cells within the foci are Tie2-positive and that they are bounded by 2 epithelia, an Ang2-expressing ependymal cell layer on the ventricular surface and, internally, by a vascular epithelium. The effects of the growth factors were also assessed by comparing the levels of Tie2 and InsRα in the ipsilateral and contralateral striatum. Ang2 showed no effect, but these optical density measurements suggest Dll4 treatment elevated expression of both the Tie2 and insulin receptors over a wide region of the monkey striatum ipsilateral to the injection site (Fig. 1*E* and Table S1). In contrast to the specific effect of Dll4 on the expression of growth factor receptors, striatal delivery of both Ang2 and Dll4 induced an increase in the number of precursor foci both ipsilateral and contralateral to the striatum that received the exogenous protein (Fig. 1*F*). These results suggest that Ang2, like Dll4, increases the numbers of precursor cells in the adult mammalian brain.

Activating Neural Precursors. The incorporation of the nucleic acid precursor bromodeoxyuridine (BrdU) into the DNA of proliferating cells is often used to assess the effects of soluble peptides on the adult nervous system. To measure numbers of proliferating cells, BrdU was delivered intraperitoneally (i.p.) to rats on days 2–4 following single injections of Dll4, Ang-2, or a combination of factors containing Dll4, Ang-2, insulin, and a JAK kinase inhibitor (combination treatment, "CT"). Insulin and the JAK inhibitor were included because they interact with Notch signaling to promote in vitro expansion of pluripotent and somatic stem cells (9, 16). At 5 days after treatment, increased numbers of BrdU-labeled cells were seen in the SVZ for all of the treatment groups, relative to control treatments that contained aCSF and BSA carrier protein (Fig. 2*A*). All 3 treatment groups showed increased numbers of proliferating cells in the rat SVZ.

To develop another measure of cellular response that did not require injection of a DNA precursor, we assessed whether a rapid change in survival signaling protein Hes3 might allow us to monitor growth factor effects in the brain. Following growth factor treatment, the number of Hes3-positive cells was clearly elevated in the adult SVZ (Fig. 2*B*; these data were obtained from every tenth section between bregma 1.70 to -0.40 mm) (20). Hes3-positive cells were not restricted to the SVZ but also found in other regions of the brain where they co-express Sox2 (Fig. 2*C*). In the striatum and substantia nigra, almost all of the Hes3-positive cells co-expressed Sox2 (97.2 $\pm$ 6.8% in striatum; 95.2 $\pm$ 11.6% in substantia nigra). However, only a small proportion of the Sox2-positive cells co-express Hes3 (12% $\pm$ 4%, $n = 4$ animals for the striatum data; 10 $\pm$ 3%, $n = 4$ animals for the substantia nigra data). We also note a 7.4-fold ($P = 0.0024$) increase in Hes3-positive cell number in the SGZ of the DG of adult rats, 5 days after a single injection of Dll4. In the SGZ, 87 $\pm$ 18% of Sox2-positive cells co-expressed Hes3 (data were obtained from every tenth section between bregma -3.14 to -5.3 mm). Sox2 plays a central role in establishing the pluripotent state of cells in the early embryo and is also required for neurectodermal differentiation (14). Three-dimensional (3D) rendering of confocal optical sections clearly shows that Hes3-positive cells have a Sox2-positive nucleus (Fig. 2*C*, inset).

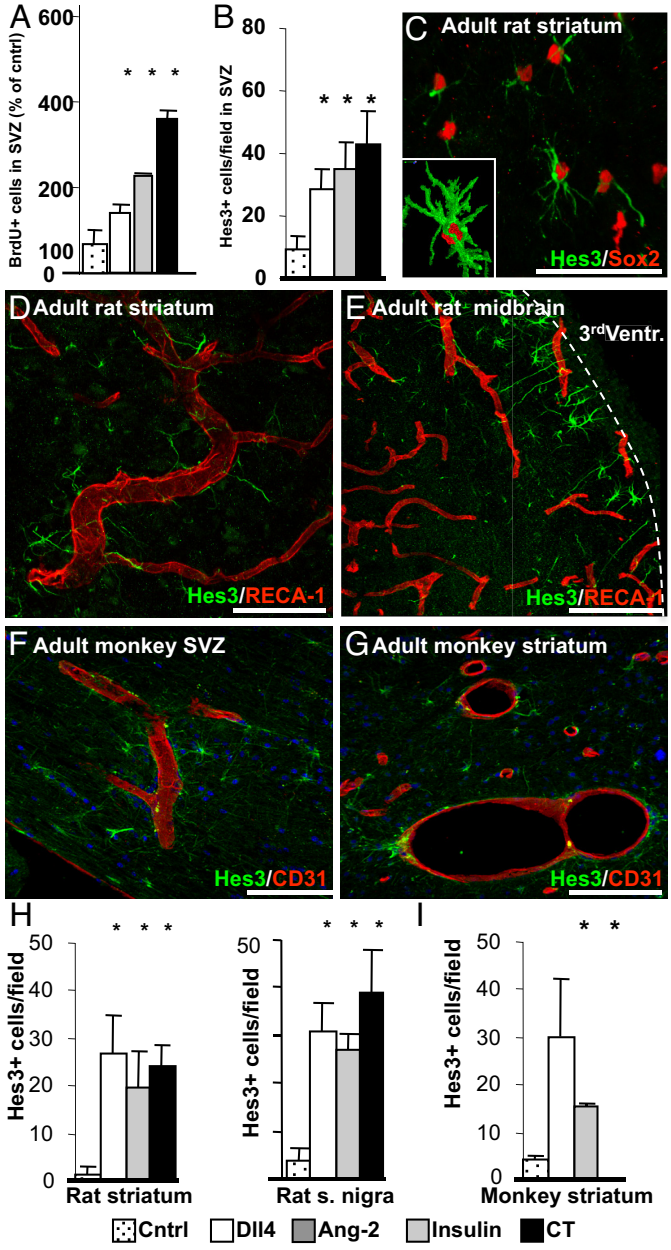

Fig. 2. Angiogenic factors activate widespread neural precursors in vivo. (*A*) Single intracerebroventricular injections of soluble factors increase $BrdU^+$ cell number in the SVZ 1 week following injection (numbers are % BSA sham injection controls) and (*B*) Hes3-positive cell number (numbers are % BSA sham controls). (*C*) Hes3-positive cells in the SVZ and striatum of control and treated animals co-express Sox2 (image from Dll4-treated striatum; inset, 3-D reconstruction from confocal z stacks). (*D* and *E*) Hes3-positive cells in the striatum and midbrain of adult rats are in contact with blood vessels (identified by RECA-1 expression; images are from CT-treated brains). (*F* and *G*) Hes3-positive cells in the SVZ and striatum of adult monkeys are in contact with blood vessels (identified by CD31 expression; images are from Ang2-treated brains). (*H*) Single intracerebroventricular injections of factors increase Hes3-positive cell number in the ipsilateral striatum and substantia nigra of adult rats (Hes3-positive cell numbers per field are shown). (*I*) Single intrastriatal MRI-guided convection delivery of factors increases the number of Hes3-positive cells in the ipsilateral striatum of adult monkeys (data are % BSA sham controls). (Scale bars: *C*, 50 μm; *D–G*, 100 μm.)

The co-expression of Hes3 and Sox2 suggests that these cells in the gray matter of the adult brain are neural precursors.

Precursor cells in many tissues are closely associated with blood vessels (21–23). Consistent with the possibility that the vascular system plays an important role in regulating precursor cells in adult tissues, Hes3-positive cells are closely associated with blood vessels in the rodent and primate brain (Fig. 2*D–G*). In this manuscript, we focus on the brain regions that contain the cell bodies and axonal projections of dopamine neurons, because a simple animal model can be used to determine the effects of growth factors on neural precursors, blood vessels, and injured neurons. A substantial increase in the number of Hes3-positive cells was seen in the striatum and substantia nigra of adult rats 5 days after intraventricular injection of Dll4, Ang2, or CT (Fig. 2*H*; $n = 4$ animals; data were generated using every tenth section between bregma 1.70 to −0.40 mm.

Hes3 expression was used to determine if a single delivery of growth factors has similar effects in the primate. MRI-guided convection was used to administer Dll4, Ang2, or BSA as a control to adult monkeys (18). Both treatments induced a small increase in the number of Sox2-positive cells in the ipsilateral striatum. In contrast, a large increase in the numbers of Hes3-positive cells was observed 10 days following a single delivery of growth factors ($n = 2$ monkeys per treatment group; Fig. 2*I*). In the monkey, the growth factors were injected ipsilaterally by convection delivery. The numbers given are a percentage of the Hes3-positive cells in the same region of the contralateral striatum. The Hes3 expression data are consistent with the increased numbers of ventricular foci and BrdU-labeled cells, suggesting that the exogenous growth factors rapidly stimulate a response in a precursor cell associated with blood vessels throughout the adult rodent and primate CNS.

Assessing Vascular Responses to Growth Factors. Signals from blood vessels are known to regulate neural development and the survival of neural stem cells (24, 25).

To determine the effects of these growth factors on the vascular system in the adult brain, the endothelial specific antigen RECA-1 was used to measure the number and size of blood vessels in the rat striatum 13 weeks after a single intracerebroventricular injection of growth factors (Fig. 3*A*; data are from sections between bregma 1.70 to −0.40 mm). Using pattern recognition software, the number and size of blood vessels could be simply assessed. Dll4 and Ang2 were demonstrated to have opposing and long-lasting effects on vessel density ("Object #"). Ang2 stimulated an increase in the size of blood vessels ("Object area"). The CT treatment did not cause a major net change in the number or size of blood vessels (Fig. 3*B* and *C*). In the adult monkey, following single injections of Dll4, Ang2, or control (aCSF containing BSA) in the striatum, the vascular response was assessed with an antibody against the vascular marker CD31. Ten days after treatment, there was no clear effect on blood vessel number, but the size of the blood vessels showed a similar response to data obtained in the rat striatum (Fig. 3*D*). These data show that long-lasting vascular changes can be caused by growth factor treatment, but suggest that combinations of growth factors minimize this effect while retaining the stimulation of neural precursors.

Growth Factors Protect Dopamine Neurons in Vivo. Since the initial serendipitous discovery of the toxicity of 6-hydroxydopamine (6-OHDA) on noradrenergic and dopaminergic neurons, this effect has been exploited in many studies (26). When limiting doses of 6-OHDA are injected into the striatum, the dopaminergic neurons in the midbrain die over the subsequent 6 weeks. At 2 weeks after the lesion, approximately half the dopamine neurons are already irretrievably injured. The rapid increase in Hes3-positive cells in the striatum and the substantia nigra raises the possibility that both the axons and cell bodies of dopamine neurons might be restored from an injured

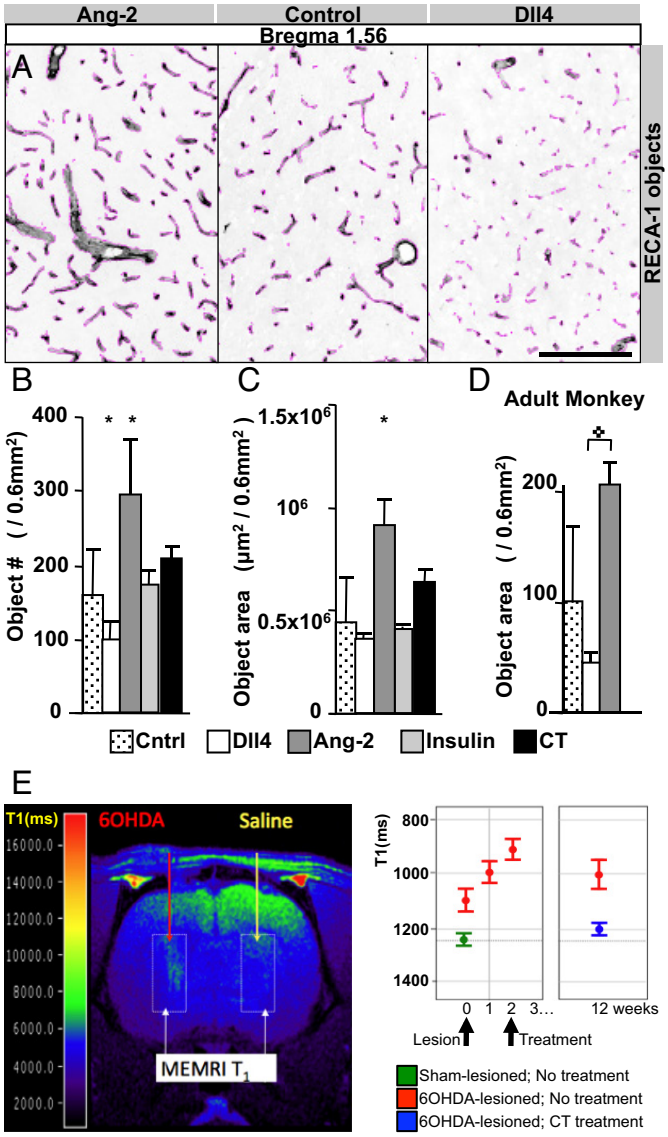

Fig. 3. Combinations of pro- and anti-angiogenic factors maintain normal vascular density. (*A*) Thirteen weeks after a single intracerebroventricular injection of Ang2, there is an increase in blood vessel number and total vessel area in the striatum of adult rats; Dll4 induces a reduction in vessel number. (*B* and *C*) Quantitation of vascular density (''object number'') and total vessel surface area (''object area'') by pattern recognition software in striatal sections stained for RECA-1. (*D*) Quantitation of total vessel surface area (''object area'') in striatal sections from adult monkeys (10 days after a single intrastriatal injection of factors), stained for CD31. (*E*) CT treatment induces the normalization of T_1 MRI signal following 6-OHDA lesion relative to sham-treated BSA controls [red bars, 6-OHDA; yellow bar, saline control (no 6-OHDA lesion); blue bars, 6-OHDA lesion followed by CT injections, 2 weeks later]. (Scale bar, 100 μm.)

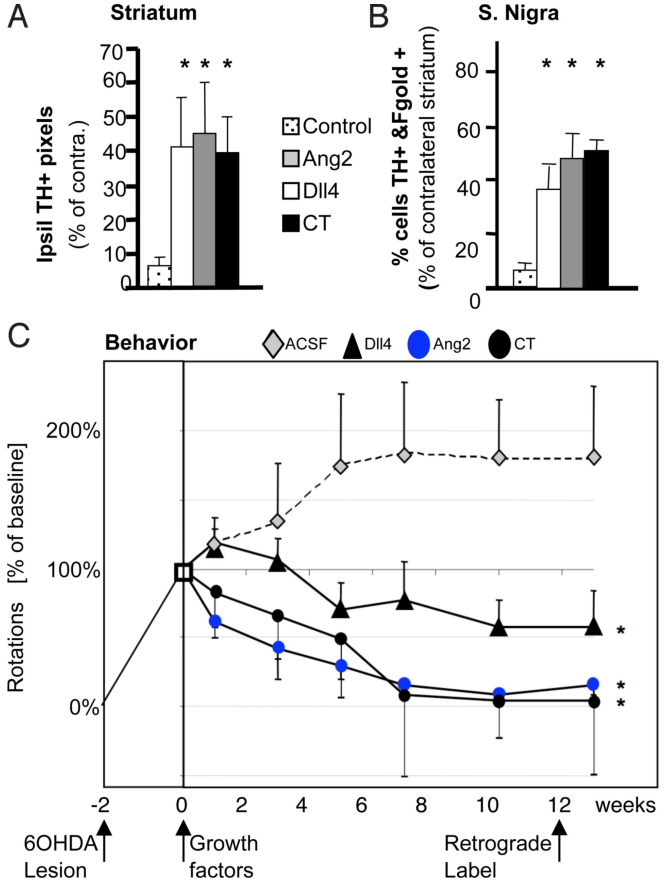

Fig. 4. Injured dopamine neurons are protected from death by single treatments with angiogenic factors. (*A*) Single intracerebroventricular injections of Dll4, Ang2, and CT result in an increase in TH^+ signal in the lesioned rat striatum (data are expressed as % ipsilateral versus contralateral TH optical density; contralateral TH signal was similar in all treatment groups). (*B*) When administered 2 weeks after a 6-OHDA lesion, the same treatments also rescue TH^+ cell bodies in the substantia nigra (assessed 13 weeks after treatment). Dopamine neurons were retrogradely labeled by fluorogold injections in the striatum, 1 week before euthanization; data are numbers of Fluorogold$^+$/TH^+ cell bodies in the substantia nigra as a % control. (*C*) Single intracerebroventricular injections of various treatments 2 weeks after 6-OHDA lesion in adult rats promote long-lasting behavioral recovery assessed by amphetamine-induced rotometry.

state by single treatments with soluble proteins to the adult brain.

Because of the concern that the vascular system would be disrupted either by the lesion or the growth factor treatment, the permeability of the blood brain barrier was measured by MRI of gadolinium chelate or manganese. Gadolinium chloride is routinely used to visualize blood brain barrier disruption, but no extravasation of gadolinium was detected in the striatum of lesioned animals. Manganese enhanced MRI (MEMRI) is more sensitive than gadolinium to small changes in blood brain barrier permeability, neuronal activity, or connectivity (27). A shortening of the T_1-weighted Mn^{2+} signal indicating an increase in vascular permeability became prominent in the central region of the ipsilateral striatum in the 3 weeks following the 6-OHDA lesion and was sustained in untreated animals (Fig. 3*E*). In contrast, elevated Mn^{2+} uptake was not observed in the T_1 image of animals treated with the combination of growth factors (CT) measured at 15 weeks after lesion ($n = 10$ rats, $R^2 = 0.72$, $P < 0.001$). These data suggest that a long-lasting subtle change in the permeability of blood vessels caused by the lesion can be corrected by exogenous growth factors.

Tyrosine hydroxylase (TH) is the rate-limiting enzyme in the biosynthesis of dopamine. The expression of TH in the striatum contralateral and ipsilateral to the lesion was used to assess the effects of growth factors on the distribution of dopaminergic axons. In the sham-operated controls injected with BSA, there were few (>5%) TH^+ processes in the striatum 15 weeks after the lesion. In all of the treated groups, in contrast, TH expression was sustained at 40% of the levels in the uninjured contralateral striatum (Fig. 4*A* and Fig. S5*a*; $n = 4$–7 rats for each group; data

were obtained from sections between bregma 1.70 to −0.40 mm). This result shows that dopaminergic projections to the striatum are rescued by single injections of soluble factors in the lateral ventricle.

Because an intact nigrostriatal circuit is a possible outcome of the therapeutic approach we report here, it is important to determine if the rescued TH^+ processes in the striatum are the projections of dopamine neuron cell bodies that reside in the substantia nigra. One week before the end of the experiment, the retrograde fluorescent tracer flurogold was injected into the striatum, and the number of labeled cell bodies projecting axons from the substantia nigra was assessed by co-expression of TH and the tracer. In the lesioned animals that received a sham injection of BSA, fewer than 10% of the dopamine neurons remained compared to the contralateral control substantia nigra (Fig. S5*b*). In contrast, all of the animals treated with growth factors retained 4-fold more dopamine neuron cell bodies in the substantia nigra (Fig. 4*B*; $n = 4–7$ rats for each group; these numbers were obtained from serial sections between bregma −4.80 and −6.30; for each section the morphology of the aqueduct provides a simple assessment of the location in the anterior-posterior axis of the brain). In unlesioned controls, 130 ± 8 TH^+/$Fluorogold^+$ cell bodies were scored in the entire area corresponding to the substantia nigra of a 30-μm section. In lesioned animals, 9 ± 3 double-positive cells were found. The number of double-labeled cells in animals treated with growth factors treated increased to 47 ± 11 for Dll4, 62 ± 12 for Ang2, and 66 ± 5 for CT. Nissl staining also confirmed the presence of neuronal cell bodies in the substantia nigra of treated animals. When BrdU was given for 3 days following treatment, no $BrdU^+$/TH^+ cells were seen in the substantia nigra. These results suggest that existing dopamine neurons in the substantia nigra are rescued from death by growth factor treatment.

Although dopamine neurons are rescued in all of the groups, there is a clear anatomical difference between the treatments. In the untreated animals and in the Dll4-treated animals, the mean area of the cell body was ≈ 150 μm^2. In the other treatment groups, the cell bodies were ≈ 300 μm^2 (unlesioned/untreated control, 170.1 ± 16; lesioned/untreated control, 139.4 ± 16; Dll4, 155.9 ± 30.7; Ang2, 271.7 ± 24; CT, 281.1 ± 26. *P* values: Dll4, 0.003; Ang2, 0.0002; CT, 0.0002). In vivo studies show that the size of the cell body in dopamine neurons is a function of their behavioral strength (28). The change in cell body size that we report here suggests that the remaining dopamine neurons are functioning more effectively after treatment with Ang2 or CT. Rats move in response to amphetamine stimulation of dopamine release and in animals where the dopamine neurons are unilaterally injured by 6-OHDA, a bias in movement toward the lesion provides an independent measure of the gradual loss of neurons. The rotational behavior measured over the 13 weeks following the treatment with growth factors shows that all 3 treated groups recovered in the rotation assay (Fig. 4*C*; $n = 4–7$ rats for each group; asterisks denote statistical significance of the treatment curves from controls as determined by 2-way ANOVA analysis). The Dll4-treated animals showed only partial recovery, while the Ang2- and CT-treated animals showed a complete recovery in rotational bias. In all of the treated groups, the behavioral recovery was stable for the entire period of observation.

Discussion

Current research toward treatments for PD depends on animal models employing cytotoxic drugs that specifically injure dopamine neurons. There is also good evidence for the role of exogenous toxins in the generation of Parkinsonian syndromes in patients (29, 30). Animal studies link dopamine neuron loss to reduced numbers of neural precursors, and patients with PD have fewer precursor cells in the hippocampus and SVZ (31). Our data demonstrate that treatments targeting neural precursors rescue injured dopamine neurons, suggesting a reciprocal interaction between neural precursor cells and dopamine neurons.

The pathology seen in PD suggests that factors outside the nigrostriatal system play an important role (32, 33). In other neurodegenerative diseases including amyotrophic lateral sclerosis (ALS), it is also clear that cell types other than the neurons with the most prominent pathology play a critical role (34). The increasing interest in the links between neurotrophic and angiogenic signals stresses the possibility that neuronal survival may be linked to angiogenesis (35). The independent regulation of neural precursors and vascular elements may have interesting implications in cancer biology, in addition to the neurotrophic effects that are the central focus of this study (36, 37). In addition, cells of the immune system may play a role in the Tie2 response and the survival of dopamine neurons (32, 38–40). Here we report a simple strategy to trigger a widespread response in neural precursors that encompass the cell bodies and the axonal projections of endogenous dopamine neurons. The role of other cells and the effects of this regenerative system in other neurodegenerative conditions can now be rapidly assessed.

Materials and Methods

Pharmacological Treatments. We used the following concentrations: Dll4 and Ang2 (in vitro, 500 ng/mL; in vivo 12.5 μg as a single injection), FGF2 (in vitro, 20 ng/mL), JAK inhibitor (in vitro, 200 nM; in vivo, 250 ng as a single injection), insulin (in vitro, 25 μg/mL; in vivo, 40 μg as a single injection), Fluorogold (0.5 μg).

In Vivo Experiments—Rodents. All animal treatments were approved by National Institutes of Health (NIH) guidelines. Male adult (3–6 months) Sprague-Dawley rats (Charles River Laboratories), weighing 250–350 g, were used for pharmacological treatments and lesion experiments.

6-OHDA Lesion. Some animals underwent unilateral lesion of the nigrostriatal dopamine pathway by stereotactical injection of 50 μg 6-OHDA into the right striatum, using the following coordinates: Bregma AP +0.5 mm, ML −3.0 mm, VD +5.5 mm (20).

Pharmacological Treatments. Unlesioned animals, or animals 2 weeks after 6-OHDA lesion, were treated with different pharmacologic agents. Five microliters of different drugs were stereotactically injected into the right lateral ventricle using the following stereotaxic coordinates: Bregma AP −0.9 mm, ML −1.4 mm, VD +3.8 mm. The following reagents were used: Dll4 (2 mg/mL), Ang2 (1 mg/mL), insulin (8 mg/mL), Jak-Inhibitor (20 μM), either alone or in combination. Animals recovered from the anesthesia and were put back into their home cages, with access to food and water ad libitum.

Labeling of Dividing Cells. Animals received i.p. injection of the tracer 50 mg/kg BrdU every 12 h for 5 days beginning on day 1 post-op to label dividing cells.

Amphetamine Induced Rotations. At multiple time points over the course of the experiment, lesioned rats were injected i.p. with 5 mg/kg D-amphetamine sulfate and placed in automated rotometer bowls. Rotational behavior was assessed by monitoring total number of whole body (360°) turns over 60 min.

MRI Imaging. MRI on rats was performed as previously described (27). Details are provided in *SI Text*.

Methods on cell culture, primate experiments, image reconstruction, reagents, quantifications, and statistical analysis are provided in *SI Text*.

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